THE UNIVERSITY OF ALBERTA

SOME ASPECTS OF THE CHEMICAL DISINFECTION OF PIPELINE MILKING MACHINES

by

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ABSTRACT

Pipeline milking plants have only comparatively recently become widely accepted and methods of testing their hygienic condition have not been standardized. In this work, two methods have been found acceptable; these involve (1) a prolonged circulating rinse of the complete plant and (2) a combined rinse and swab of the pipeline with a separate rinse of the milking clusters.

In designing a method for cleaning and disinfecting milking equipment there are three factors to be considered in addition to the requirement of obtaining satisfactory cleanliness and bacteriological conditions. These are inexpensiveness, quickness and virtual elimination of the personal factor. Until now, the only method of cleaning a pipeline milking plant in place was by a circulation cleaning method. This method is time-consuming unless automatic controls are installed but such controls are quite expensive for the average farmer. A new method of cleaning pipeline milking plants in place, termed 'lye flooding',

has been developed. This method involves flooding the line and clusters with 5% sodium hydroxide solution containing 0.25% ethylenediaminetetra acetic acid for the complete time between milkings. So far, the bacteriological results from this method compare favourably with those from the circulation cleaning method.

During the course of this study considerable disparity was noted between the numbers of bacteria recovered from (a) swab and (b) rinse tests of pipelines. Evidence is given that some testing methods only remove a small fraction of micro-organisms from milk contact surfaces. This raises the question of the origin of the majority of bacteria in raw milk and questions the validity of our existing tests of the hygienic quality of milk contact surfaces.

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INTRODUCTION

At the beginning of this century the only agents used for the disinfection of milk contact surfaces were steam and hot water. In the 1920's chemical disinfection was introduced and chlorine was used in the form of sodium and calcium hypochlorite. While hypochlorites are still used to-day, other chemical disinfectants have also gained acceptance, viz. quaternary ammonium compounds (QACs), iodophors, mixed halogens and detergents with disinfectant properties such as acidified wetting agents and sodium hydroxide. For equipment in the dairy and food industries, chemical disinfectants must satisfy a number of requirements. Since it is quite likely that small amounts of disinfectant will be left on surfaces, these materials must be relatively non-toxic, non-tainting and non-corrosive.

Before satisfactory chemical disinfection of a surface can be accomplished, the soiling material

must be removed by a detergent. Removal of the soil and the disinfecting procedure may be done as separate operations or may be combined by the use of detergent-disinfectants.

A brief account of the principles involved in these procedures is given in the following sections.

DETERGENTS AND DETERGENCY

NATURE OF DEPOSITS TO BE REMOVED FROM MILK CONTACT SURFACES BY THE DETERGENT.

The chemical composition of detergents may be varied according to the type of surface to be cleaned and the nature of the deposit to be removed. The nature of the deposit on milk contact surfaces depends partly on the method of handling the milk. On surfaces which come in contact with cold milk, there remains after rinsing a film of butterfat and small amounts of soft protein material, principally casein. When hot milk comes into contact with simi-

lar surfaces, the deposit obtained is more difficult to remove. As the temperature rises, increasing amounts of inorganic materials, principally calcium phosphates, as well as protein and fat are deposited on the surface. For the removal of this type of deposit the detergent is usually formulated from a number of compounds.

CONSTITUENTS OF DAIRY DETERGENTS

Inorganic dairy detergents may be divided into two main groups - alkaline and acid.

The principal constituents of alkaline detergents are sodium hydroxide, sodium carbonate, tri-sodium phosphate and sodium silicates. Surface active agents and sequestering agents may also be incorporated. As all the main constituents possess an alkaline reaction, these detergents act by dissolving the protein and also reacting chemically with the fat, forming soaps. There are three main types of surface active agents, namely, anionic, cationic and non-ionic agents. The anionic and non-ionic agents when dissolved in water will remove

fat and they are included with the alkalis to supplement the detergent properties. The use of cationic surface active agents, such as quaternary ammonium compounds will be dealt with later as they are more outstanding as bactericides rather than detergents. Sequestering agents are also incorporated, the two principal types being sodium phosphates and organic amine derivatives e.g. ethylenediaminetetra acetic acid (EDTA). These materials are particularly effective in the removal of deposits from hot milk contact surfaces.

The principal acid detergents are HNO_3 and H_3PO_4. On milk contact surfaces they are usually used as a separate treatment after the alkali treatment as they are effective in the prevention or removal of inorganic deposits such as milkstone and those from hard water. The greatest disadvantage in the use of acids is their corrosiveness to metals.

GENERAL PRINCIPLES OF DETERGENCY

Most detergent processes occur in four stages

as follows:

(i) Wetting of the surface of soiling material;
(ii) Separation of the soil from the surface that is being cleaned;
(iii) Dispersion of the soiling material in detergent;
(iv) Prevention of re-deposition of soil.

Stage I. Wetting of surface

Surface active agents such as soaps are used for wetting the surface of the soiling material. The wetting power of a surface active agent owes its ability to reduce the surface tension between the liquid and the surface of the soiling material so that instead of forming droplets the liquid will spread rapidly. According to Fieser & Fieser (1956), all surface active compounds contain a hydrophylic and hydrophobic group, preferably at opposite ends of the molecule. In this way a bond is formed between the oil or fat in the soiling material and the aqueous detergent solution.

Stage II. Removal of soiling material from surface that is being cleaned

In this stage the soil may be chemically altered by the detergent either causing it to be more easily removed or the detergent may spread beneath the soil and dislodge it. Niven (1955) pointed out that to effect the final removal of the soil from the surface being cleaned, some degree of mechanical action is necessary for almost all detergent processes, though he may not have considered soaking procedures taking several hours.

Stage III. Dispersion of soiling material in detergent

The detergent used in this stage is varied according to the type of soil and according to the detergent property required, such as hydrolysis, emulsifying and suspending power etc. In this stage also, mechanical energy is usually required.

Stage IV. Prevention of redeposition of soil

In this stage the detergent solution containing the soil may be continually agitated or it may be removed and the surface immediately rinsed, thus removing any soil which might again become deposited.

DISINFECTANTS

PROBABLE MODE OF ACTION OF CHEMICAL DISINFECTANTS

All living cells contain a large number of enzymes and the metabolic cycle of each cell consists of the total of all the reactions catalyzed by its enzymes. Enzymes exhibit the properties of proteins and some have been shown to be pure proteins while others have a non-protein part attached to the protein moiety. As a protein structure constitutes at least part of the enzyme it follows that physical or chemical means which de-nature proteins will also inactivate enzymes. This fact is considered to be the probable explanation of the bacteriostatic and bactericidal action of disinfectants. The disinfectant may inactivate one or more of the enzymes, thus causing a break in the metabolic cycle of the organism.

A number of factors affect disinfection, including concentration of disinfectant, contact time, temperature of disinfectant, presence of organic matter, pH, initial concentration of organisms, state of dispersion of organisms, type of surface involved, water hardness and the inclusion of surface active agents in the disinfectant. Those of the above variables which are particularly important and subject to variation in the disinfection of milk contact surfaces are discussed further in the following pages.

PRINCIPAL FACTORS AFFECTING DISINFECTION

Concentration of disinfectant

A logarithmic relationship exists between the concentration of disinfectant and the rate of disinfection, varying with each disinfectant, e.g. if the concentration of phenol is doubled the reaction velo-

city may be increased sixty four times, while doubling the strength of mercuric chloride increases it only twice (Fairbrother, 1959). The rate of disinfection is also dependent on the extent and rate of penetration of the disinfectant into the cell, so it is possible with some disinfectants that an increase in concentration may not give a corresponding increase in activity. According to Sykes (1958) a 1% solution of phenol is lethal for many bacteria in a few minutes but a 0.5% solution requires several hours for the same effect. Weber & Levine (1944) studying the germicidal efficiency of chlorine on Bacillus metiens spores reported that when the concentration of available chlorine was doubled the disinfection time was reduced by 45 - 50%.

Contact time

The process of disinfection is not instantaneous but a gradual procedure and as a result the contact time of the disinfectant on the organisms is of paramount importance. Whitehouse (1961) suggested that

disinfectants do not produce an immediate kill because of variation in susceptibility of individual cells. This variation in susceptibility is in keeping with normal individual variation in any biological population and is probably mainly a result of the changes in the enzymic makeup of the cell. With chemical disinfection of milking equipment, Clegg (1962) pointed out that the contact time between the disinfectant and the milk contact surface is probably the most important of the variables affecting the chemical disinfection of these surfaces.

Presence of organic matter

The presence of organic matter, especially protein, lowers the activity of most disinfectants; according to McCulloch (1945) sodium hydroxide is least affected. Cox & Whitehead (1949) studied the bactericidal action of chlorine in the presence of protein and found that the chloramino compounds formed by interaction of hypochlorite and protein or amino acids have a slower disinfecting action against

Streptococcus cremoris than free hypochlorite. Sykes (1958) suggested that the lowering of germicidal activity of disinfectants in the presence of organic matter may be caused by physical protection or may be of a competitive nature as a result of a similarity in structure of some of the cell constituents with those in the organic matter.

Temperature

Temperature also affects the velocity of reaction, an increase in temperature usually enhancing the activity of the disinfectant. High temperatures alone can effect disinfection through heat inactivation of enzymes. At temperatures below that of the denaturation of protein, disinfection is a chemical reaction and a rise in temperature will increase the velocity of the reaction, though not to the same degree with all disinfectants. Fairbrother (1959) pointed out that with a rise of $10^{o}C$ the reaction velocity of phenol is increased eight times while that of mercuric chloride is increased two to four times. It is possible to conceive in a situation where the organisms to be dis-

infected are growing in a suitable medium, an increase in temperature up to the optimum temperature for growth may not enhance the rate of disinfection because of the attainment of more suitable conditions.

McCulloch, Hauge & Migaki (1948) reported that the germicidal activity of QACS is depressed by low temperatures. Barber (1949) noted that in general a rise in temperature resulted in a shortening in killing time but the extent of the effect varied with the particular QAC.

pH

Hydrogen ion concentration is an important variable with some disinfectants. Johns (1948) found that pH in the range 10.0 - 5.0 influenced the germicidal activity of hypochlorites even more than an increase in the available chlorine content from 50 to 100 p/m, the germicidal activity being enhanced when the pH was lowered from above 10 to below 7. Wolf & Cousins (1946) showed on the other hand that vegetative cells of bacteria in dried

milk films are destroyed less rapidly by neutral than by alkaline solutions of hypochlorites, though this did not apply to spores of bacteria and in the instances cited with non-spore forming bacteria the result is almost certainly one of increased detergent action on the milk film making the disinfectant more available. Swartling (1949) found that the germicidal effectiveness of some QACs is much greater at pH 10 - 11 than at lower pH levels.

INTERACTION OF DETERGENTS AND DISINFECTANTS

Recently more use has been made of the knowledge that some detergents possess disinfecting properties and *vice versa*. An example of the former is NaOH which owes its disinfecting property to its high alkalinity. In fact Davis (1956) has rated the disinfecting power of some alkaline detergents according to decreasing alkalinity as follows:

$$NaOH > Na_2CO_3 > Na_3PO_4 > Na_2CO_3 . NaHCO_3 .$$

Examples of a disinfectant possessing detergent properties are (a) the QACs and (b) acidified wetting agents. With QACs the detergent properties are probably not great in the small concentrations of use as disinfectants (100-200 p/m). However, with the acidified wetting agents the materials are used at *ca*. 1600 p/m which is bound to have some detergent effect; the disinfecting property is somehow bound up with the use of a surface active material at a low pH (3.5).

As well as the 'alternative' characteristics of detergents and disinfectants it is known that in

some circumstances, they posses synergistic properties. Examples of these are (a) the enhanced cleaning properties of a chlorinated tri-sodium phosphate in comparison with 'straight' T.S.P. (Cox & Whitehead 1949) and (b) the greater disinfecting properties of a mixture of sodium hypochlorite and sodium carbonate as against sodium hypochlorite alone against dried milk films (Neave & Hoy, 1947).

For these reasons detergent-disinfectants, which have been known and used for two decades, are becoming more popular. With pipeline milkers there is probably no time-saving advantage of using detergent-disinfectants as there is with bucket milkers. However the synergistic effect of the two materials may make this type of material more popular as might the fact that manufacturers undoubtedly prefer to supply some materials ready mixed rather than run the risk of having operators misuse products by mixing together incompatibles i.e. cationic and anionic materials, etc.

PIPELINE MILKING PLANTS

The purpose of pipeline milking plants is to convey the milk from the milking machines to the milk house thus avoiding the necessity for manual transportation and possible contamination of the milk. The milk contact surfaces involved are usually glass, stainless steel and rubber.

Although the first pipeline milking plants were installed in the 1920's, it was not until recent years that they have been widely accepted. All the early pipelines were installed in milking parlors, but today when construction of a pipeline milking plant is contemplated, there is a choice between a stanchion barn installation and a milking parlor. The principal differences between these two types of installation are that with parlors the pipelines are much shorter and the cows are brought to the milking machines, whereas with the stanchion barn the milking machine clusters must be manually moved from cow to cow.

With each of these installations, the method of

transferring the milk from the line to the milk house is by means of vacuum and a vacuum releaser, which may be actuated by gravity or a pump or may be a vacuum bulk milk tank.

The pipelines may be made of stainless steel or glass, the majority being part glass and part stainless steel. The advantage of glass is that the effectiveness of cleaning can be judged by visual inspection without dismantling the line.

Most of the pipelines until the early 1950's were of the milking parlor type as it was necessary to dismantle the line for cleaning after each milking. With the advent of cleaning in place (CIP) dismantling after each milking became unnecessary and longer pipelines were installed. There are two types of pipeline milker arrangements - single and double line. The single line arrangement which is used in single row stanchion barns or parlors, consists of a single line to the terminal end of the barn or parlor. A double line arrangement forms a continuous circuit around a parlor or double row stanchion barn.

Some of the systems for the cleaning in place of pipeline plants are outlined below:-

(a) Vacuum Re-circulation. This is designed for double line systems where the CIP solutions, which may or may not be mixed with air, are drawn by vacuum through the pipeline system, returning to the circulation unit located in the milk house.

(b) Vacuum Gravity. This is designed for single lines and the cleaning solutions are repeatedly drawn through the pipeline system to a tank at the terminal end by the vacuum and on release of the vacuum are allowed to return to the original reservoir in the milk house by gravity.

(c) Reverse Vacuum. This is designed for single lines. The method is similar to that of (b) except that the solutions are returned to the reservoir in the milk house by vacuum.

(d) Pressure System. Circulation of the CIP solutions is obtained by forcing them through the pipeline system by a pump of sufficient capacity to produce effective cleaning.

The system used on the majority of pipeline milking plants is the vacuum re-circulation method as single systems are usually converted to double lines by installing a cleaning solution return line which is used for washing and disinfecting purposes only.

SOME ASPECTS OF CIRCULATION CLEANING OF PIPELINE MILKING PLANTS

The most widely used circulation cleaning method involves a cold or tepid water rinse of the plant immediately after milking, followed by a hot alkaline detergent wash for 15 - 30 min after which the plant is again rinsed with water. Immediately before the next milking the plant is disinfected. Once a week the plant receives an acid treatment and the milking machines are dismantled and brushed-washed. With many pipeline milking plants, especially in Britain, a similar routine is practiced, except that the washing and disinfecting procedures are combined by the use of detergent-disinfectants.

Cleaning aspects

Temperature

In most chemical reactions a rise in temperature increases the rate of the reaction. Thus, as the action of the detergent on the soiling material in pipelines is partly a chemical reaction, it follows that a rise in temperature will increase the effectiveness and rate of cleaning. Jennings (1959) established from a series of cleaning trials with a circulating fluid at different temperatures and for a particular set of conditions that a linear Arrhenius relationship exists between temperature change and soil removal. Calbert (1958)found that satisfactory cleaning could be attained in a laboratory circulation unit with the detergent temperature at the start of the washing cycle, 130 - 140^{o}F and dropping to 90^{o}F by the end.

The statement that a rise in temperature increases the effectiveness of cleaning may not hold true for all detergents, as some surface active agents

become less soluble at high temperatures and are partially deposited. (Society of Dairy Technology 1959). Various detergent temperatures for circulation cleaning have been used and recommended by several workers. Since most of the work was carried out from the bacteriological point of view, the question of detergent temperature is dealt with further under bacteriological aspects.

Turbulence

In the case of circulation cleaning of pipeline milking plants, turbulence constitutes the mechanical action referred to under Stage II of the general principles of detergency. With brush washing, the mechanical energy is supplied by the movement of the brush, but in circulation cleaning the energy must be produced by friction between the detergent solution and the soiling material. The friction is obtained by the turbulence of the solution which is produced by a high rate of circulation and by the incorporation of air. Jennings, McKillop & Luick (1957) concluded from experiments on films of milk solids that further

increases in turbulence over flow conditions equivalent to a Reynold's Number of 25,000 improve the effectiveness of cleaning. A speed of not less than 5 ft/sec is generally recommended for circulation cleaning and the usual size of milk pipelines is $1\frac{1}{2}$ in. diam. A flow of 5 ft/sec in a $1\frac{1}{2}$ in. diam. line is equivalent to a Reynold's Number of approximately 100,000 which is four times the minimum suggested by Jennings et al. (1957). The incorporation of air, although it increases the turbulence, may also be a limiting factor due to foam formation. Some detergents, particularly those containing surface active agents tend to form a foam which then protects the soiling material from the detergent.

Bacteriological aspects

The final objective common to all circulation cleaning of pipeline milking plants is, with due regard to time and cost, to render the milk contact surfaces clean and as free as possible from microorganisms. The complete sequence of operations, i.e. rinsing, washing and disinfecting is designed to achieve this goal.

The rinsing operations, as well as removing milk or detergent residues, also get rid of micro-organisms which are loosely attached to the inner surfaces. Good rinsing will remove up to 90% or more of the organisms which have been left on a plant with which cold milk has come in contact (Ashton, 1961). The detergent wash undoubtedly removes with the soiling material many organisms adhering to the plant surface, and the disinfecting operation is intended to kill the majority of the remaining organisms.

With regard to the efficiency of these operations in attaining satisfactory bacteriological conditions, the question of temperature again arises. The main problem is, whether or not chemicals must be supplemented by 'bactericidal' heat in the form of hot detergent or detergent-disinfectant solutions, hot water rinsing or steaming.

Cuthbert, Bird & Bateson (1954) obtained satisfactory results from twice daily flush-washing a releaser plant with a detergent solution containing 500 p/m hypochlorite at 160°F, followed by a warm

water rinse. The plant was steamed weekly. Twice weekly steaming gave better results, but when steaming was carried out once every two weeks the results were unsatisfactory.

Thornborrow (1960) studied circulation cleaning methods of pipeline plants which had been adapted for this type of cleaning. The circulation temperature varied from 'cold' to 170°F and she found that where temperatures greater than 130°F were used, the bacterial count was lower.

Cuthbert (1960) in discussing the bacteriological problems arising from circulation cleaning suggested that the application of heat, together with modifications in plant design were necessary.

Murray, Downey & Foote (1962) made a comparison of two methods of circulation cleaning, one method involving a detergent wash and disinfection by steam and the other a combined detergent-hypochlorite treatment. The initial circulation temperature in both cases was 150 - 165°F. They report comparable bacteriological results for rinse and milk samples with both methods. Thomas

(1963) stated that circulation of a detergent-disinfectant at an initial temperature less than 140°F did not provide adequate cleansing. Snudden, Calbert & Frazier (1961) using the separate washing and disinfecting method, made a bacteriological study of in-place cleaning of a pipeline, the detergent solution being circulated for 22 - 24 min at the following temperatures: (i) 150 - 160°F; (ii) 160°F at the beginning, falling naturally to about 90°F; (iii) 125°F at the beginning, falling to about 93°F. They found that the bacteriological condition of the pipeline was satisfactory at all detergent temperatures, the Standard Plate Count of the milk averaging below 20 x 10^3 organisms/ml during each test period.

Mueller (1962) in a study on cleaning and disinfecting a portable milk transfer unit including 100 ft of plastic pipeline, reported that it was readily maintained in a satisfactory sanitary condition by the use of a chlorinated alkaline cleaner circulated for 15 min with a minimum start and finish temperature of 130° and 115°F. He also reported that when an ordinary dairy cleaner was

used in place of the chlorinated alkaline cleaner and the plant was given a disinfectant treatment after the cleaning operation, it could not be maintained in a satisfactory sanitary condition.

It would appear from the above reports that in order to render a circulation cleaned pipeline milking plant bacteriologically satisfactory, chemicals must be supplemented by some form of heat treatment, but there is lack of agreement as to the extent of this treatment. The lack of agreement may be attributed to a number of factors including equipment design, types and condition of surfaces involved, climatic conditions and methods of testing.

EXPERIMENTAL AND DISCUSSION ON EXISTING METHODS OF CIRCULATION CLEANING AND TESTING OF PIPE-LINE MILKERS

DESCRIPTION OF PLANT USED FOR TESTS

The results in this thesis were obtained from tests on the pipeline milking plant at the University Farm, which is a stanchion barn installation with a round-the-barm pipeline. The plant consisted of 4 milking units, 250 ft of 1.5 in. diam. glass pipeline and 12 ft of 1.5 in. diam stainless steel line. (Glass piping sizes are determined by the internal diameter and stainless steel piping by the outside diameter.) The pipeline was connected to a 500 gal. vacuum bulk milk tank.

Two milking units from each of two manufacturers, Surge and De Laval, were used. A glass breaker jar connected the inflations to the long milk tube in the Surge machine (Plate 1), whereas with the De Laval machine a stainless steel claw piece was used (Plate 2). With the De Laval machine the filter was incorporated in the long milk tube, whereas with the Surge machine, it was placed within the breaker

PLATE 1. Surge milking machine cluster showing glass breaker jar.

PLATE 2. De Laval milking machine cluster showing claw piece
and in-line filter holder.

jar. The long milk tubes in both cases were 8 ft long, those of the De Laval machine were made of plastic while the Surge units were made of rubber.

Ninety ft of the glass part of the pipeline was installed by Surge and the remainder of the glass line, the stainless steel part of the line and the circulation cleaning arrangement were put in by De Laval, the main differences between the two sections of pipeline being the type of milk inlets and type of couplings used for connecting sections of the line.

During milking the long milk tubes were connected to metering devices which were joined by a short tube to the milk inlet. The milk inlets on the Surge section of the line consisted of 0.5 in. diam. holes with a rubber ring around the circumference. A stainless steel slide arrangement covered each hole forming a hermetic seal with the rubber ring except when the inlet was in use during milking. To connect the milking machine to a milk inlet, the slide was moved back and a stainless steel nipple, attached to the end of the short tube connected to the milk meter, was placed over

the inlet (Plate 3). In the De Laval part of the line, there were 6 in. stainless steel sections of tubing from each of which a 2 in. milk inlet protruded, which tapered from 0.8 in. to 0.6 in. diam. at the end. The connection of the milking machines to these inlets was made by sliding the short tube from the meter over the end. Except when milking, these inlets were hermetically sealed by rubber caps (Plate 4).

The connections between sections of both lines had similar neoprene gaskets. The Surge couplings were made from stainless steel whereas plastic couplings were used on the De Laval glass section and stainless steel couplings on the stainless steel section (Plates 5 & 6).

The circulation cleaning procedure was automatically controlled. The basic components of the automatic washer arrangement were the control panel and the wash unit. The control panel incorporated the electrical relays and timer controlling the various washing and disinfecting cycles. Both

PLATE 3. Surge sliding connection to milk inlet.

PLATE 4. Rubber seal on De Laval milk inlet for circulation cleaning.

PLATE 5. De Laval plastic coupling.

PLATE 6. Surge stainless steel coupling.

washing and disinfecting cycles could be varied. The wash unit held the switches, water solenoid valves, mixing valve, detergent chamber and water diversion valve (Plate 7).

RECOMMENDED MATERIALS AND METHODS FOR TESTING THE EFFICIENCY OF DISINFECTION OF MILK CONTACT SURFACES

Rinse and swab contact methods are recommended by the American Public Health Association (1960) and the (British) Ministry of Agriculture & Fisheries (1955), for checking the efficiency of disinfection of dairy equipment. Rinse methods are commonly used for equipment with relatively small surface areas, such as bottles and cans. Swab contact methods on the other hand, are applied to equipment involving irregular or large surface areas which are not suitable for rinsing, e.g. bulk milk tanks. Swabs are also particularly useful in locating localized sources of contamination.

The rinse solution recommended by the American Public Health Association (1960) is 0.00425% potassium dihydrogen phosphate. Where a chemical disinfectant has been used it is necessary to include a neutralizer

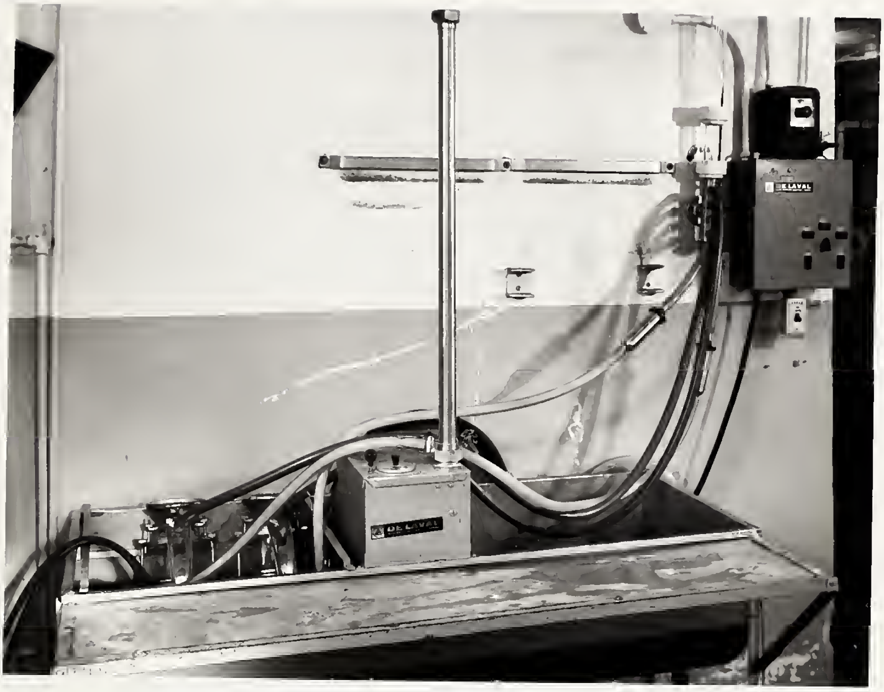

PLATE 7. Control panel, wash unit and wash trough for circulation cleaning.

for that particular disinfectant.

The recommended swabbing materials are non-absorbent cotton wool or calcium alginate wool, the recovery solution for the cotton swab being the rinse solution above, and that for the alginate wool consisting of the same rinse solution with 2% sodium hexametaphosphate.

The rinse solution recommended in Britain is quarter strength Ringer's solution incorporating a disinfectant neutralizer where necessary. The recommended swabbing material is non-medicated cotton gauze, the recovery solution consisting of the rinse solution above.

These rinse and swab methods do not remove all the organisms from the surfaces tested and consequently a negative result does not mean that bacteria are absent. The usefulness of these methods lies in the fact that they give an indication of failures in the washing or disinfecting procedures.

TRIAL METHODS FOR TESTING THE SANITARY CONDITION OF PIPELINE AND MILKING MACHINE CLUSTERS

Rinse of both pipeline and milking machine clusters

Since there was no standard procedure which could be used to assess the hygienic condition of pipeline milking plant several ideas were tested to see which gave the most reliable results.

The line and clusters were rinsed by circulating through the plant, 20 gal of tap water to which 10 ml of sterile 10% (w/v) sodium thiosulphate solution had been added. Tests were made immediately before the disinfection rinse in the afternoon, the plant having received a detergent wash after the morning milking. Tests were also made after disinfection, with contact times of 3 and 7 min. To obtain a 3 min contact time, 20 gal of disinfectant was drawn through the line once, whereas circulation of the disinfectant for 5 min gave a contact time of 7 min. The rinse water was circulated for different periods of time. The results are shown in Table 1. In all cases the control count/ml of the rinse water containing sodium

TABLE 1. Results of bacteriological rinses of both pipeline and milking machine clusters following different treatments

Treatment of plant	First 2 gallons of water through plant		Rinse water circulated for 15 min		Rinse water circulated for 30 min	
	Colony count (/ml)	Calculated no.of organisms removed from plant (10^3)	Colony count (/ml)	Calculated no.of organisms removed from plant (10^3)	Colony count (/ml)	Calculated no.of organisms removed from plant (10^3)
Not disinfected	45	4091				
Not disinfected	52	4728	240	21,820	-	-
Not disinfected	-	-	100	9,092	150	13,638
Disinfected (contact) time 3 min)	-	-	140	12,728	170	15,456
Disinfected (contact time 3 min)	-	-	48	4,364	8	647
Disinfected (contact time 7 min)	-	-	40	3,637	63	5,728
Disinfected (contact time 7 min)	-	-	100	9,092	50	4,546

thiosulphate before use was found to be negligible.

From the results it appears that with this method of testing the hygienic condition of a pipe-line milking plant, circulation of the rinse water for 15 min gave as good if not a better indication of the bacteriological condition of the plant than circulating for 30 min or testing the first of the rinse through the plant.

A disadvantage of testing the first 2 gal of water through the plant is that much of it has not formed a solid 'plug' of water through the complete line but has spread out, rinsing only the lower internal surface of the pipe. It has been suggested that the bacteriological condition of the first milk through the line gives an indication of the condition of the equipment. This appears to be reasonable, judging from the results obtained from the first 2 gal of rinse water. However, it should be remembered, especially in cases where the plant is disinfected immediately before milking, that any disinfectant which has not drained from the line comes through with the first milk.

This may give unreliable results as the disinfectant not only dilutes the milk but quite likely has an antibacterial effect.

Day-to-day variability in the bacteriological condition of the plant is a factor which may account for the variation in results following the same treatment.

As the results obtained after 15 min circulation of the rinse water appeared to give as good an indication of the bacteriological condition of the plant as the other two tests, it was decided to use this circulation time for the tests in the following section.

Comparison of rinses and swabs of pipeline and milking machine clusters

Comparisons were made between rinses and swabs of the pipeline and milking machines. One ft^2 of the line was swabbed before rinsing, through a milk inlet by means of a cotton gauze swab on a flexible holder. The number of organisms obtained from this one ft^2

was taken as the average/ft^2 for the complete line and the total number of organisms for the whole line was calculated. The four milking clusters (two clusters of each type) were used in the test. One inflation from each cluster, one claw piece and one breaker cup were swabbed with cotton swabs. The number of organisms obtained from each liner was considered to be the average for each liner on the cluster to which it was attached, while the result from the claw piece was taken as half the total for both claw pieces, and that from the breaker cup as half of total for both breaker cups. The total for the sixteen liners, two claw pieces and two breaker cups was then calculated. The line, without the clusters was rinsed by circulating for 15 min, 20 gal of tap water to which had been added 10 ml of sterile 10% sodium thiosulphate. The long milk tubes were used for connecting the pipeline to the wash trough in which the rinse water was collected. A sample of the rinse water was taken before and after rinsing for 15 min. The clusters were then included in the circuit for another 15 min, after which another sample was taken. The samples were tested by the

colony count method on Petri dishes after incubation at 32°C for 48 hr and the bacterial count for the line and clusters calculated. The results in Table 2 were obtained by the above methods following different treatments of the plant after morning milking.

TABLE 2. Comparison between rinses and swabs of pipeline and milking machine clusters with different treatments

Treatment of pipeline	Colony count (10^3) from			
	Pipeline		Clusters	
	S P	R P	S M	R M
A	371	20,000	36	25,000
B	239	20,000	120	14,000
C	8,000	23,000	85	12,000
D	41	30,000	8	46,000
E	47	111,000	90	46,000
F	36	61,000	74	17,000

SP Calculated number of organisms for complete pipeline from results of swab of 1 ft^2.

RP Count from rinse of complete pipeline.

SM Calculated total number of organisms for 4 milking machines from results of swab of 1 inflation from each plus 1 claw piece and 1 breaker cup.

RM Count from rinse of 4 milking machines.

<u>Treatments</u>

A Line and clusters washed for 20 min with 0.5% tri-sodium phosphate and disinfected with sodium hypochlorite 200 p/m available chlorine for 3 min.

B Line and clusters washed for 20 min with 0.5% tri-sodium phosphate and disinfected - 200 p/m available chlorine for 7 min.

C Line and clusters washed for 20 min with 0.5% tri-sodium phosphate and disinfected - 20 p/m available chlorine for 3 min.

D Line and clusters washed for 20 min with 0.5% tri-sodium phosphate and disinfected - 20 p/m available chlorine for 7 min.

E Line and clusters washed for 20 min with 1% tri-sodium phosphate.

F Line and clusters washed for 20 min with 0.5% tri-sodium phosphate.

It is fairly clear from the results in Table 2 that there is little variation between the results of the rinse tests in spite of intentional differences in composition of treatment solution. There are somewhat greater differences between the swab results (apart from one outside value) but these differences do not tie in with the treatment differences. The most striking difference is between the swabs and the rinses. Generally speaking, the results from the rinses are 1,000 times greater than the swabs. Two explanations can be suggested which might contribute to this difference: (1) the rinse was circulated for 15 min before sampling, and this represents more vigorous scrubbing of the milk contact surface than the mere rubbing of a swab for a few seconds; (2) the swabs were made only on the smooth internal surface of the pipeline, whereas the rinse would cover the entire surface, including areas where milk solids might be expected to accumulate such as around the gaskets between sections of the pipeline.

The swab of the pipeline for treatment F using

only 0.5% tri-sodium phosphate does not afford a good comparison with the swab of treatment A, 0.5% TSP followed by 200 p/m sodium hypochlorite. However, the swab of F gives a 'good' result of 36,000 bacteria calculated for the whole of the pipeline, i.e. $333/ft^2$ and one would not consider that this result could be improved greatly, even with the addition of disinfectant to the detergent solution. In other words, 20 min wash with 0.5% TSP is a most effective treatment. There is some doubt as to whether the higher results from swabs A and B represent a significant difference from swab F. However, it is apparent that an increase in the physical cleaning of the plant yields more bacteria as evidenced by the vastly increased numbers in the rinse solutions which were circulated for 15 min. Certainly the swab F took off only a small proportion of the total microorganisms present in the pipeline.

In addition to the above considerations, the results of the clusters do not constitute a satisfactory comparison of the swab and rinse methods. Before these tests were made it was assumed that

the number of organisms in the long milk tube would be negligible in comparison with those in the inflations, but later tests showed otherwise.

Although it appeared from the results that this method of rinsing a pipeline milking plant was reasonable, it had a number of limitations. If the water used were not of consistently good bacteriological quality it would require a bactericidal treatment. If a chemical bactericide were used, the excess would have to be neutralized before making the test. This method of rinsing is also time-consuming and it would only be used where circulation cleaning is practiced. There is also a possibility of contamination of the rinse water from the air because of the proximity of the barn. Further, the outside of the teat cups if contaminated during milking would be bound to affect the final result. For the above reasons the rinse was not chosen as the preferred method of testing. However, at that time, the striking differences between swab and rinse results were not appreciated. Had these been realized a different testing method might have been developed from the one

eventually chosen.

Comparison of rinses and swabs of inflations

As inflations are considered to be the most probable source of contamination in milking machines, it was decided to make some comparisons under standardized conditions between cotton gauze swabs, alginate wool swabs and rinses, on milking machine inflations. In examining tests to determine the hygienic condition of milk contact surfaces or the efficiency of different disinfectants or detergent-disinfectants in practice with use dilutions, it is necessary to resort to exaggerated soiling of the surfaces to be treated in order to have the best possible chance of differentiating between test methods or agents which are designed to give satisfactory results (low bacterial counts) at use dilutions. Accordingly such a soiling method was used for the clusters. Soiling was done by drawing through the inflations and breaker jar 1 gal of aged milk containing approximately 20 x 10^6 organisms/ml.

The counts of the milk were obtained by the

direct microscopic method modified by Levine & Black (1948) and recommended by the Americal Public Health Association (1960). The milk adhering to the outside of the teat cups was hosed off with tap water, the inflations were allowed to dry at room temperature for 2 hr and were then rinsed with 1 gal of tap water in a manner similar to the soiling procedure. Any excess water was shaken from the inflations and immediately the interior of one inflation was swabbed with a cotton gauze swab, another with an alginate wool swab and a third was rinsed. The recovery solution for the cotton swab was 25 ml of $\frac{1}{4}$ strength Ringer's solution and for the alginate wool swab 25 ml of $\frac{1}{4}$ strength Ringer's solution to which 2 ml of sterile 20% sodium hexametaphosphate solution had been added immediately before testing to dissolve the alginate wool; each inflation was swabbed twice. The rinse solution consisted of 500 ml of $\frac{1}{4}$ strength Ringer's solution. The inflation was rinsed by placing the mouth of the bottle over the large opening in the inflation and, holding the short milk tube doubled, the inflation was rinsed by inverting both bottle and inflation twice while they were maintained in the position described. Duplicate colony counts of these rinses were made and

the average results of these tests are shown in Table 3.

TABLE 3. Results from comparisons of cotton swab, alginate swab and rinse of similarly treated inflations

Colony counts (10^3)/inflation obtained by:		
Cotton swabs	Alginate swabs	Rinses
Surge Flat Dome		
3.0	2.6	2.0
4.4	1.2	23.5
4.4	8.0	13.0
1.8	3.0	1.0
De Laval		
7.2	25.0	67.5
2.0	30.2	31.5
176.0	3.4	38.0
47.2	11.4	109.5
28.8	26.2	24.0
Surge Orange Line		
9.8	0.8	1.0
0.2	0.6	0.5

The results in Table 3 were obtained from three different types of inflations. Both of the Surge types were made from a rubber and synthetic mix while the De Laval inflations consisted of natural rubber alone. The Surge-Flat Dome and the De Laval types were both narrow bore and contained a groove immediately inside the teat opening. The Surge Orange-line is a wide bore type and does not contain a groove, the teat opening being concave.

From the results it can be seen that there is considerable variation within each method of testing. It would appear in the case of the Surge Flat Dome type that the cotton swabs gave the most consistent results but that the greatest recovery was obtained by the rinses. With the De Laval inflations the rinse gave the most consistent results and yielded the greatest recovery of bacteria. There was little difference between the methods in the case of the Surge Orange-line type of inflation except for one result with the cotton swab.

From the overall results it would appear that the rinse is the best method for assessing the hygienic condition of milking machine inflations.

Comparison of rinse of one inflation with rinse of complete cluster

Tests were carried out to check if under standardized conditions the result obtained from rinsing one inflation gave a reproducible indication of the bacteriological condition of the complete cluster. To obtain standard conditions the cluster was soiled with milk containing 20 x 10^6 organisms/ml and allowed to dry and rinsed in the manner previously described. One inflation was rinsed twice with 500 ml of $\frac{1}{4}$ strength Ringer's solution and the remainder of the cluster was rinsed as follows. The cluster was placed upright with the open end of the long milk tube above the level of the cluster and the inflations held perpendicular by means of a wooden stand (Plate 8). Each of the 3 inflations was rinsed with 500 ml of $\frac{1}{4}$ strength Ringer's solution by compressing the short milk tube, filling the inflation with the rinse solution and allowing it to empty into the breaker jar. This procedure was repeated on each of the 3 inflations. The rinse solution was then swirled and collected in a flask through the long milk tube. The averaged results obtained

PLATE 8. Stand used for bacteriological rinsing of milking machine clusters.

from duplicate plates are given in Table 4.

TABLE 4. Comparison of rinse of one inflation with rinse of complete cluster

Colony counts (10^3) on complete cluster	single inflation	Ratio Cluster: inflation
1387	132	10:1
4640	140	33:1
3028	68	44:1
4329	114	38:1

All these results show reasonable reproducibility when the count/ml of the soiling medium is taken into consideration. So, it would appear that the method of soiling the equipment is satisfactory.

However, it cannot be assumed from these results that, when testing a milking machine cluster without intentional soiling, a rinse of one inflation will give an indication of the complete cluster.

Swab of entire pipeline by means of alginate wool

This method involved swabbing the entire milk contact surface of the pipeline with an alginate wool swab which was drawn through the line by means of vacuum. The swab consisted of a sterile 5 g plug of alginate wool which was dampened with distilled water and sterilized in a glass jar. The swab was drawn through the line into 2 litres of recovery solution in a stainless steel container. This container was 6 in. diam. and 12 in. high and was completely enclosed except for two 2.5 in. length of stainless steel pipes (1.5 in. diam.) welded to the top. One of these pipes was connected to the vacuum line and the other to the milk line at the milk discharge point by means of a rubber sleeve (see Plate 9). The outlet connected to the vacuum line contained a cross bar for checking the swab to prevent it being drawn into the vacuum line. The recovery solution consisted of 2 litres of $\frac{1}{4}$ strength Ringer's solution containing 2 g sodium thiosulphate and 40 g sodium hexametaphosphate. When the above method was first tried, there were 2 sharp right angle corners

in the pipeline which tended to break up the swab. When these corners were replaced by right angle sweeps the swab did not break up in the line as often as previously.

The line was soiled by drawing through it 2 gal of milk with a high bacterial count. The milk was then allowed to 'dry-on' for various times after which it was rinsed with 2 gal of tap water to remove excess milk and leave only that adhering to the surface of the line. Two alginate swabs were used, the first being dissolved in the recovery solution and a sample taken before the second was drawn through the line into the same recovery solution. The counts of the soiling milk were obtained by the direct microscopic count and of the swab recovery solution by duplicate plate colony counts.

Although numerous tests were carried out to check the effect of time of drying and also to compare the results when the line was soiled with milk of differing bacterial content, it was possible to obtain results from only a few tests because of the disintegration of the swabs. These results are set

out in Table 5.

TABLE 5. Results of using alginate wool for swabs of pipeline

Count of milk used for soiling line (10^6)	Drying time (hr)	Count from complete swabs (10^6)		Total from 2 Swabs
		First	Second	
40	1.5	11	25	36
40	4	72	-*	>72
40	6	16	29	45
8	0	4	8	12

* Swab disintegrated

Swabbing the line with alginate wool and dissolving the swab in the recovery solution appeared to be a promising method but it is difficult to draw conclusions from the results obtained because of the lack of duplicate results. It would appear that more useful soiling was done with high count milk and a drying time than low count milk without a drying time. Drying time in excess of 1.5 hr did not appear to affect the outcome. For this reason and because it was

PLATE 9. Stainless steel container used for recovering rinse solution from line showing connections to the milk and vacuum lines.

necessary to wash and disinfect the plant before the afternoon milking, a drying time of 2 hr appeared suitable for succeeding tests. It also appeared that one swab removed a sufficient number of organisms to give an indication of the condition of the line. It was found difficult to prepare swabs of the same dimensions, this would affect the pressure each swab exerted against the internal surface of the pipeline. A possible explanation for the high result from the test with the 4 hr drying time may have been that the swab exerted greater pressure against the milk contact surfaces than in the case of the other tests.

Attempts were also made to swab the line without intentional soiling with high count milk but only one result was obtained. A count of 3×10^6 organisms was obtained from a swab 5 hr after the detergent wash following morning milking.

Some tests were also made with the swab tied by means of cotton thread. In this case it did not break up in the line but it would not dissolve in the recovery solution as a type of gel formed on the outer surface, and even after 24 hr the recovery solution had not pene-

trated through the gel. Tying the swab with a thread made from alginate wool was then tried but the same difficulty was encountered. Varying the concentration of sodium hexametaphosphate in the recovery solution was also tried but without success. It was then decided to abandon the use of an alginate wool swab.

Combined rinse and swab of pipeline

When the above tests were run it was noticed that the swab removed any solution which had not drained from the line. This water formed a solid plug ahead of the swab with the result that the line received a rinsing and swabbing action at the same time. It was decided to check if, under standardized conditions, this type of rinse gave a reproducible indication of the bacteriological condition of the line.

The line was soiled by drawing through it 2 gal of milk containing approximately 20 x 10^6 organisms/ml. After 2 hr drying time it was rinsed in the same way

with 2 gal of water. With these tests a sponge was used as the swab. Before sterilization approximately 100 ml of water was incorporated in the sponge which was then wrapped in aluminum foil and autoclaved. The sponge was placed in the line and the outlet of the stainless steel container with the cross bar was connected to the milk line, and the other outlet to the vacuum line. When vacuum was applied, before the sponge moved through the line, the water was extracted from the sponge thus forming a small plug of water, and as the sponge progressed, more water which had not drained from the line was collected. This water was mixed with 2 litres of sterile $\frac{1}{4}$ strength Ringer's solution containing 2 g sodium thiosulphate in the stainless steel container. The sponge which was trapped by the cross bar in the outlet of the container was transferred by means of sterile tweezers to a flask containing 1 litre of sterile $\frac{1}{4}$ strength Ringer's solution and 1 g sodium thiosulphate. The number of organisms obtained from the line by both rinses and swabs were estimated by duplicate plates. The results obtained are given in Table 6 together with results

TABLE 6. Tests with combined rinse and swab method after soiling line with milk containing approximately 20 x 10^6 organisms/ml followed by a 2 hr drying

Treatment	Colony counts (10^3) from complete line from		
	rinse solution	swab	rinse plus swab
I. Rinsed with 2 gal tap water	54,000 32,000 69,000 62,000	4,000 1,000 4,000 6,000	58,000 33,000* 73,000* 68,000
II. Rinsed with 20 gal water, washed with 0.5% tri-sodium phosphate for 20 min and a-gain rinsed with 20 gal water	6,000 10,000	416 409	6,416 10,409
III. Treatment II and disinfection with sodium hypochlorite (200 p/m available chlorine) for 3 min	696	31	727
IV. Treatment II and dis-infection with sodium hypochlorite (200 p/m available chlorine) for 7 min	147	1	148

* These results obtained on same day

after washing and washing plus disinfecting procedures after the line had been soiled as already described.

The differences in results from treatment I (Table 6) are not great when the count of the soiling milk is taken into consideration. The tests giving total counts of 33×10^6 and 73×10^6 were made on the same day by delaying afternoon milking. After running the first test the line was washed (i.e. treatment II), after which it was again soiled, allowed to dry for 2 hr, rinsed with 2 gal of water and tested. When these tests were made it was not realized that so many organisms would remain on the surfaces after treatment II. Consequently, it is possible that the result of 73×10^6 organisms would have been lower if the test had been made on a different day or if the plant had been disinfected as well as washed following the preceeding test.

The results obtained following treatments III & IV indicate the necessity for disinfecting as well

as washing a plant which is bacteriologically unsatisfactory. It also appears in the case of unsatisfactory plants, that contact time of the disinfectant makes an appreciable difference.

Because of the satisfactory reproducibility of the results following treatments I & II and because the results after treatments III & IV were in accord with expectation, it was decided to test this method further with other detergents and detergent-disinfectants with a view to using it for comparing methods of cleaning-in-place.

USEFULNESS OF THE COMBINED RINSE AND SWAB WITH DIFFERENT DAIRY DISINFECTANTS AND DETERGENT-DISINFECTANTS

In order to test the usefulness of the combined rinse and swab method for pipeline milkers it was decided to try out the method with artificially soiled pipeline treated with different disinfectants and detergent-disinfectants. Before doing this it is perhaps desirable to review the different methods which have been used for evaluating detergents and

disinfectants as the method used might eventually be developed as a method of evaluating such agents specifically for pipeline milkers.

As the practical application of such tests involves the destruction of micro-organisms adhering to surfaces, except of course in the case of water disinfection, it follows that a test under practical conditions or which simulates these conditions is the most desirable. Clegg (1955) pointed out that the method for evaluating a disinfectant should closely approximate the proposed method of use and this is particularly so with the disinfection of dairy utensils.

A method described by Johns (1947) for comparing the disinfecting efficiency of QACs. and hypochlorite products involved dipping a sterile microscope slide in a skim milk and water mixture inoculated with a test organism. The slides were allowed partially to dry and were then immersed in the disinfectant, agitated gently for 1 - 20 sec depending on the test, rinsed, placed in Petri dishes,

flooded with agar and incubated. Johns found that QACs were more effective against Gram-positive bacteria such as *Staphylococcus aureus* than the Gram-negative, whereas hypochlorites were equally effective against Gram-negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa* as against Gram-positive organisms.

Goetchius & Botwright (1950) used a similar method for the evaluation of QAC detergent-disinfectant formulations except that they used rubber strips in place of the glass slides.

Garvie & Clarke (1955) made a comparison of the effectiveness of QACs and hypochlorites against bacteria adhering to the surfaces of stainless steel strips with the effectiveness of these materials against bacteria in the disinfectant solution. They found that the organisms inoculated into a disinfectant solution were more easily destroyed than when they were adhering to a surface.

Neave & Hoy (1947) in a study of the disinfection of contaminated metal surfaces with hypo-

chlorite solutions used tinned trays which were artificially infected with diluted milk containing <u>Staphylococcus</u> <u>aureus</u>. This work which was commenced in 1941 was probably the first method of testing detergent-disinfectant solutions on contaminated surfaces at 'use dilution'.

Hoy & Clegg (1953) developed a can test for the screening of detergent and detergent-disinfectant formulations. The test involved soiling of 10 gal cans with aged milk of known quality and allowing the cans to drain, and stand overnight. The cans were then treated with the test material for a specified time. The treated surfaces were rinsed with $\frac{1}{4}$ strength Ringer's solution, the rinsing being supplemented by the use of a 'squeegee'. The Ringer's solution was then plated and aliquots added to fresh raw milk to observe the effect on keeping quality. The test materials were compared against a mixture of sodium carbonate and sodium hypochlorite.

Cousins, <u>et</u> <u>al</u>. (1960) reported a comparison between suspension tests and can tests and considered that suspension tests may be of some value for screen-

ing QAC-detergent mixtures but with other disinfectants e.g. hypochlorite and iodophors, the suspension test may be less reliable.

Tests under practical use conditions have also been used for evaluating the bactericidal efficiency of disinfectants and detergent-disinfectants. These tests are usually termed farm trials. Clegg et al. (1959) tested a number of formulations which had given satisfactory results with the can test by means of farm trials and obtained a close relationship between these two methods.

Johns (1962) compared the effectiveness of an iodophor for washing and disinfecting milking equipment with a chlorinated alkaline detergent plus hypochlorite by means of farm trials. He reported that, with both procedures over 80% of the initial plate counts were $<$10,000/ml, and he concluded that with adequate cleaning procedures strong concentrations of the disinfectant are unnecessary.

FURTHER TESTS OF COMBINED RINSE AND SWAB METHOD WITH ARTIFICIALLY SOILED PIPELINE TREATED WITH DIFFERENT DISINFECTANTS AND DETERGENT-DISINFECTANTS

Soiling the line and milking machine clusters was accomplished with milk containing approximately 20×10^6 of naturally occurring bacteria/ml, allowing the milk to dry on for 2 hr and then treating the milk contact surfaces with a disinfectant or detergent-disinfectant solution. The numbers of micro-organisms in the milk was determined by a direct microscopic count. The procedure was as follows. One gal of the contaminated milk was placed in each of 2 pails. Two clusters were connected to the line and the milk was drawn through them and the pipeline by means of vacuum. After 2 hr drying the machines were placed in the wash trough to which 20 gal of the disinfectant solution was added. The disinfectant solution was drawn through the clusters and the line to give a contact time of approximately 3 min. The milking machine clusters were then each rinsed with 1 litre of $\frac{1}{4}$ strength Ringer's solution. The line was tested by the combined rinse and swab method, the recovery solution for the rinses being

1 litre and for the swabs 500 ml of $\frac{1}{4}$ strength Ringer's. To the rinse solutions for the clusters and the recovery solutions for the combined rinse and swab test was added a neutralizer for the particular disinfectant used.

Some of these tests were made when only the 2 Surge machines were in use. When the De Laval machines were also available one of each type was used.

This method of testing is somewhat similar to the Hoy can test. The length of drying time is necessarily cut short because after testing, the plant had to be washed and disinfected before the afternoon milking. With the can test, the can is rotated at 1 rev/sec during treatment with the detergent-disinfectant, the milk film thus receiving a certain scrubbing action. In the opinion of Johns (1962) this scrubbing action is not sufficient to place it on a par with the vigorous brushing needed for manual cleaning of 'dried on' milk residues on milking equipment. Clegg (1955) pointed out that, as most of the materials tested are detergent-

disinfectants the can test was used to obtain a measure of detergency as well as disinfectant action, and the materials were compared with a satisfactory control consisting of a solution of sodium hypochlorite and sodium carbonate. With the method developed here for the pipeline milking plant the milk film received a vigorous scrubbing action due to the speed and turbulence of the detergent and detergent-disinfectant solutions.

The disinfectants tested were sodium hypochlorite, and Roccal which is the trade name for alkyl dimethyl benzalkonium chloride (ADBC). The recommended use dilutions for these disinfectants were 200 p/m for both sodium hypochlorite and Roccal.

The detergent-disinfectants tested were Iosan which is an iodophor, Saf-Sol which is a hypochlorite-hypobromite detergent-disinfectant and Diochem which contains sodium hypochlorite, sodium meta-silicate and tri-sodium phosphate. The recommended use dilutions for disinfection purposes were solutions containing 12.5p/m available iodine for Iosan, 100 p/m available halogen expressed as chlorine for Saf-Sol

and 100 p/m available chlorine for Diochem.

The neutralizer used for the chlorine and iodine containing disinfectants and detergent-disinfectants was sodium thiosulphate. In the cluster rinse solutions and in the recovery solutions for the swab and rinse of the pipeline 0.05% and 0.1% sodium thiosulphate was added respectively. With the QAC solutions 0.4% Asolectin and 1% Tween 80 was added to the cluster rinse solutions and 1% Asolectin and 1% Tween 80 was added to the recovery solutions. The results of these tests which are averages of duplicate counts are shown in Table 7.

It is known that the results of disinfection tests are liable to be erratic (Sykes, 1958) and in order to overcome this, tests require adequate replication. In work with a pipeline plant in a dairy barn it is necessary to complete one day's experimental work in time for the next milking. This places a limitation on the number of replicate determinations which can be done at one time and seldom can more than one test be done in one day.

It would clearly be unwise to make too much

TABLE 7. A comparison of the effectiveness of different disinfectants and detergent-disinfectants for pipeline milkers

Material	Colony count (10^3) on					
	Rinse of pipeline	Swab of pipeline	Rinse and swab of pipeline	Rinse/cluster with		
				Surge No.1	Surge No.2	De Laval
DISINFECTANTS						
Sodium hypochlorite						
200 p/m available chlorine	2.6	0.4	3	1	30	-
200 p/m " "	2.5	- *	>2.5	80	44	-
100 p/m " "	9	1	10	7	3	-
100 p/m " "	6.5	0.5	7	78	16	-
Roccal						
200 p/m ADBC	140	4	144	22	-	33
200 p/m "	75	38	113	29	-	248
DETERGENT-DISINFECTANTS						
Saf-Sol						
100 p/m available halogen	140	3	143	236	49	-
100 p/m " "	486	102	588	117	-	148
50 p/m " "	2,511	10	2,521	58	135	-
Iosan						
25 p/m available iodine	197	42	239	534	429	-
12.5 p/m " "	895	58	953	98	13	-
Diochem						
100 p/m available chlorine	147	12	159	276	-	216
50 p/m " "	528	57	585	69	-	57

* Accident

of the differences shown in Table 7 as it was not possible, under the conditions of working, to do sufficient replicate tests to arrive at an estimate of the error of a single determination.

The conclusion to be drawn from Table 7 is that because replication of tests on one day is impossible, it would be necessary in order to compare two different treatments to run each treatment for a considerable number of days and make daily tests and regard these as a type of replicate test which could be compared with a similar series using a different treatment.

CLEANING IN PLACE

One of the purposes behind this work was to assess a new proposed method for cleaning and disinfecting pipeline milkers in place. To do this not only was it necessary to have a method to determine the hygienic condition of a pipeline, but also to have a standard treatment with which the experimental treatment could be compared. The standard reference treatment chosen was a vacuum re-circulation method using an alkaline detergent and sodium hypochlorite according to the following method.

CIRCULATION CLEANING INVOLVING SEPARATE WASHING AND DISINFECTING PROCEDURES

This method was used in the University barn on the pipeline milking plant from the time it was installed in March 1962 until the end of January 1964. The milking machine clusters to which air bleeders

were attached were placed in the wash trough and connected to a manifold at the end of the line. The other end of the milk pipeline and the vacuum line were attached to the top of a 5 gal stainless steel receiver. The bottom of the receiver was connected to the wash unit through a pump containing a check valve. The solutions, mixed with air were drawn by vacuum from the wash trough through the milking machine clusters and pipeline to the receiver from which they were pumped to the wash unit. During the detergent washing operation the solution was returned to the original reservoir from which it was re-circulated and during the rinsing and disinfecting operations it was run to waste (Diagram 1).

(See back of page for key to Diagram 1)

(To face page 77)

KEY TO DIAGRAM 1

1. Wash trough
2. Wash unit
3. Clusters
4. 500 gal vacuum bulk milk tank
5. Receiver
6. Pump
7. Sanitary trap
8. Vacuum cock
9. Vacuum pump
10. Drain valve

DIAGRAM I

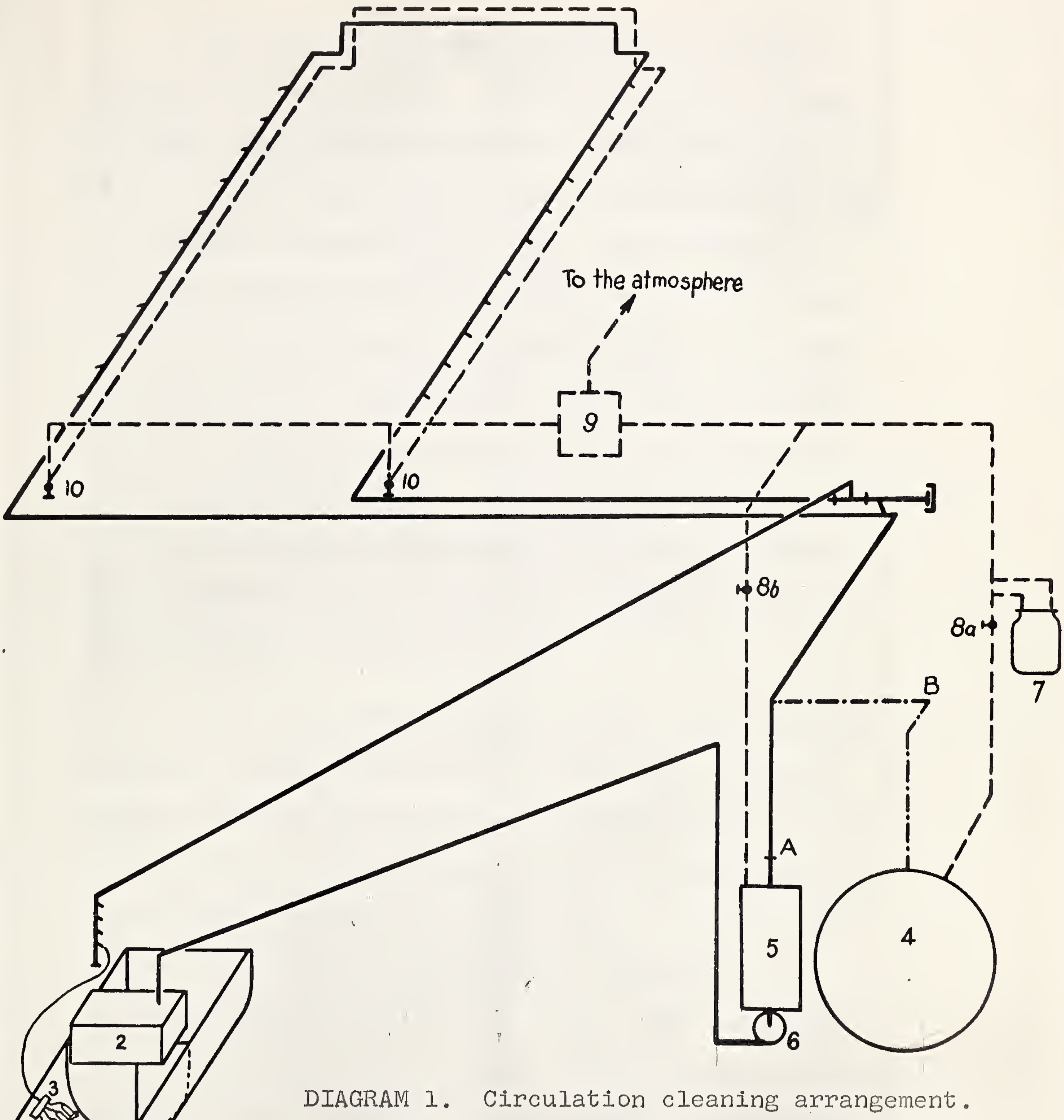

DIAGRAM 1. Circulation cleaning arrangement.

____________ Milk and cleaning solution line.

------------ Vacuum line.

The complete sequence of operations between milkings was as follows: Immediately after milking, the line and clusters were pre-rinsed with 20 gal of water at 85°F which was followed by washing with 0.5% tri-sodium phosphate for 20 min. The detergent solution before circulation began was at 140°F and at the end of 20 min had dropped to 95°F. The line and clusters were again rinsed with 20 gal of water at 85°F. Twice a week the post-wash rinse was replaced by an acid rinse. Immediately before the next milking the equipment was disinfected with a sodium hypochlorite solution containing 200 p/m of available chlorine at 85°F for 3 min.

Once a week the milking machines were disassembled and soaked in an acid solution for 4 hr after which they were brush-washed in a detergent solution before being re-assembled. Two sets of inflations were used in rotation for each cluster. Each set, after being used for one week, was immersed in 5% sodium hydroxide solution for 6 hr. They were then dry stored until the next week. The stainless steel section of the line was dismantled once every 2 weeks, soaked in an acid solution and brush-washed with a detergent solution.

Bulk milk tank

When the pipeline was first installed a 300 gal stainless steel atmospheric type bulk milk tank was in use. In October 1962 this was replaced by a 500 gal stainless steel vacuum tank.

The atmospheric type tank was manually cleaned and disinfected. The cleaning process consisted of rinsing immediately after the milk was removed followed by brush-washing with an iodophor (25 p/m available iodine). The tank was disinfected immediately before milking, again with iodophor (12.5 p/m available iodine) which was sprayed over the entire inner surface by means of a spray arrangement which was attached to the water hose. The tank received a once weekly treatment with milk stone remover.

The vacuum tank was rinsed with tap water immediately after removal of the milk and was then washed with 0.5% tri-sodium phosphate for 30 min by a recirculation spray method. This arrangement consisted of a pump which was connected to the outlet of the tank and also to a spinner which was suspended inside the

tank. The spinner had small holes in it and the pressure from the pump turned the spinner thus forming a spray which made contact with the complete interior of the tank. The tank was disinfected immediately before milking with sodium hypochlorite (200 p/m available chlorine) for 5 min, also by means of the re-circulation spray method. The tank was treated once a week with milk stone remover.

Both bulk milk tanks were tested by the cotton swab technique, the recovery solution being 25 ml of $\frac{1}{4}$ strength Ringer's solution containing 0.05% sodium thiosulphate. The base, agitator and outlet in the atmospheric tank and the inside wall and outlet in the vacuum tank were swabbed. With the vacuum tank it was not possible to reach the agitator with the swab. The results obtained are given in Tables 8 & 9.

TABLE 8. Results of swab tests on atmospheric bulk milk tank

Date	Colony count on Base ($/ft^2$)	Agitator ($/ft^2$)	Outlet ($0.2\ ft^2$)
24/4/62	25	75	-
30/4/62	75	25	300
8/5/62	25	50	580
14/5/62	25	75	180
22/5/62	75	1930	75
28/5/62	25	25	125
7/6/62	75	130	280
11/6/62	25	25	130
19/6/62	75	25	180
25/6/62	280	100	380
17/7/62	50	75	9300
28/8/62	1680	50	180
5/9/62	25	50	430
11/9/62	25	25	25
17/9/62	25	50	1650
27/9/62	50	25	50

TABLE 9. Results of swab tests on vacuum bulk milk tank

Date	Colony count on	
	Inside wall ($/ft^2$)	Outlet ($0.2\ ft^2$)
8/11/62	1500	1000
16/12/62	25	630
18/ 2/63	100	50
18/ 6/63	500	100
4/ 7/63	25	150
18/ 7/63	1400	630
2/10/63	25	50
6/ 1/64	180	100
17/ 2/64	300	880
27/ 2/64	50	50
10/ 3/64	25	50
23/ 3/64	75	130

As a colony count of $5000/ft^2$ is usually considered satisfactory it would appear from the results in Tables 8 & 9 that the methods used for cleaning and disinfecting both tanks were satisfactory. Jensen et al. (1959) compared a manual method of cleaning with a re-circulation spray method using a chlorinated detergent in both cases and found the bacteriological condition of the tank after spray cleaning was better than that after manual cleaning. There appeared to be no appreciable difference in the present work between the manual and spray methods (Tables 8 & 9).

Judging from the results in Table 2 it is probable that the counts in Tables 8 & 9 are a very small fraction of the micro-organisms on the surfaces tested. It would be possible to check this in the case of the vacuum tank by using the spinner washer for circulating a rinse solution, thus making it possible to rinse the entire inside surfaces of the tank for a prolonged period.

CLEANING PIPELINE MILKERS IN PLACE BY THE LYE FLOODING METHOD

Lye flooding of pipeline milkers involves the complete filling of the clusters and pipeline with 5% sodium hydroxide solution containing 0.25% ethylenediaminetetra acetic acid (EDTA) so that the interior surfaces are in contact with it for the complete time between milkings. The method is analagous to that of immersion cleaning developed by Thiel et al. (1955, 1956) using sodium hydroxide solution and EDTA for direct-to-can milkers.

Immersion cleaning involves the complete immersion of the clusters and other suitable parts in the lye-EDTA solution in specially designed containers for the whole of the time between milkings. Immediately before milking the equipment is removed from the immersion solution and rinsed with chlorinated water (50 p/m available chlorine). After milking, any outside dirt is removed from the equipment which is again rinsed in the same chlorinated water used before milking to remove traces of milk before further immersion. Once a month the clusters are dismantled,

brush-washed in a detergent solution and liners replaced if necessary. At the same time the lye-EDTA solution is renewed.

This method when properly used has been found to give satisfactory results in England (Thiel et al. 1955, 1956, Carreira et al. 1955, Sheppard 1959) and in Canada (Whitehouse & Clegg 1961). It is also recommended by the Ministry of Agriculture, Fisheries and Food (1961) in Britain.

So far as is known the lye flooding method at present in use at the University Farm is the first such C.I.P. system to be applied to a pipeline milking plant. The adapted plant is shown in Diagram 2 and the procedure is as follows.

Immediately after milking the clusters are suspended from specially designed racks in the rinsing tank (2) and connected to the line by means of a manifold. When the milk has drained from the line, vacuum cock 5b is closed, the coupling at point B is disconnected and the one at point A is loosened. The

(See back of page for key to Diagram 2)

(To face page 87)

KEY TO DIAGRAM 2.

1. 45 gal lye-EDTA tank
2. 30 gal rinsing tank
3. Clusters
4. 500 gal vacuum bulk milk tank
5. Vacuum cocks
6. Vacuum line draining valve
7. Sanitary traps
8. Inlet for rinse water hose
9. Vacuum pump
10. 3 gal receptacle
11. Air inlet

DIAGRAM 2

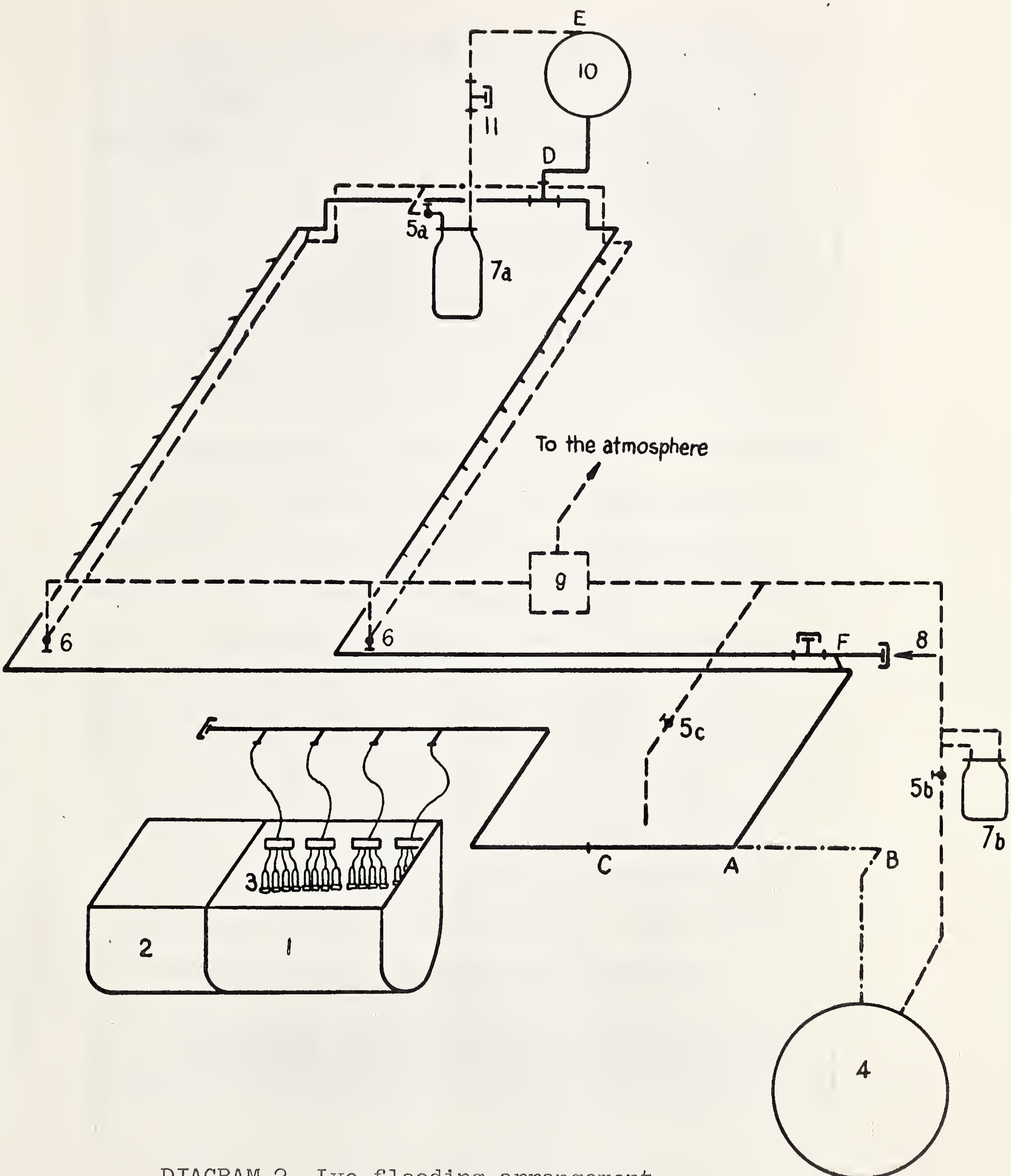

DIAGRAM 2. Lye flooding arrangement.

__________Milk and cleaning solution line.

---------Vacuum line.

coupling B is then connected at point C and the coupling at point A is again tightened. A 1.5 in. outside diam. water hose which is connected to a 1 in. water main is inserted in the line at point 8 and pushed beyond the connection between the two lines (F). The plant is then rinsed for 5 min by forcing the water through the line and clusters after which it is allowed to drain for 15 min.

The clusters are next suspended in the lye-EDTA solution in tank 1 (Plate 10). A rubber stopper is placed in the line at point 8, the vacuum cock at position 5a is opened and the vacuum pump is switched on. The lye-EDTA solution is drawn by vacuum through the clusters and line to receptacle (10). When the receiver is half-full the vacuum cock at position 5a is closed and the vacuum pump is switched off. When the line is completely filled sufficient lye-EDTA solution must remain in the tank so that the ends of the teat cups are below the surface. Thus, the line and clusters remain flooded between milkings.

Immediately before the next milking the line is drained by opening the vacuum cocks at positions 5c

and 5a. This breaks the vacuum and the solution flows back into the tank and the line is allowed to drain for 15 min. Each cluster is then taken from the lye-EDTA solution, allowed to drain and suspended in the rinsing tank. The vacuum cock at 5a is closed and the line and milking machines again rinsed and allowed to drain as already described. The coupling at position C is disconnected and the line again connected at B. The section of line from B to the bulk milk tank is brush-washed with a detergent and disinfected with sodium hypochlorite (200 p/m available chlorine) for 5 min.

Once a month the milking machines are disassembled, soaked in an acid solution for 4 hr and brush-washed in a detergent before being re-assembled. At the same time the lye-EDTA solution is renewed.

When converting the plant from circulation cleaning to the lye flooding method, it was necessary to change the type of seal on the De Laval milk inlets. With circulation cleaning the seal was obtained by means of a rubber plug (Plate 4) and for lye flooding

adaptation the seals consist of rubber caps which slide over the ends of the inlets and are clamped in position (Plate 11). It was also necessary to tighten the slides on the Surge milk inlets as some of them were not forming hermetic seals. This was not observed when the circulation cleaning system was in use as small leaks are not revealed due to the speed and turbulence of the solution and the fact that air is already incorporated in the solution through the air bleeders in the clusters.

When the plant was first modified for lye flooding the receiver (10) was not installed and a 1.5 in. diam. line connected points D and E. This gave rise to a large air bubble remaining in the high section of the line because the sanitary trap (7a) filled with lye-EDTA solution, thus cutting off the vacuum before all the air was removed from the line.

COMPARISON OF LYE FLOODING OF PIPELINE WITH CIRCULATION CLEANING USING ALKALINE DETERGENT AND SODIUM HYPOCHLORITE

For the purposes of a comparison of both the

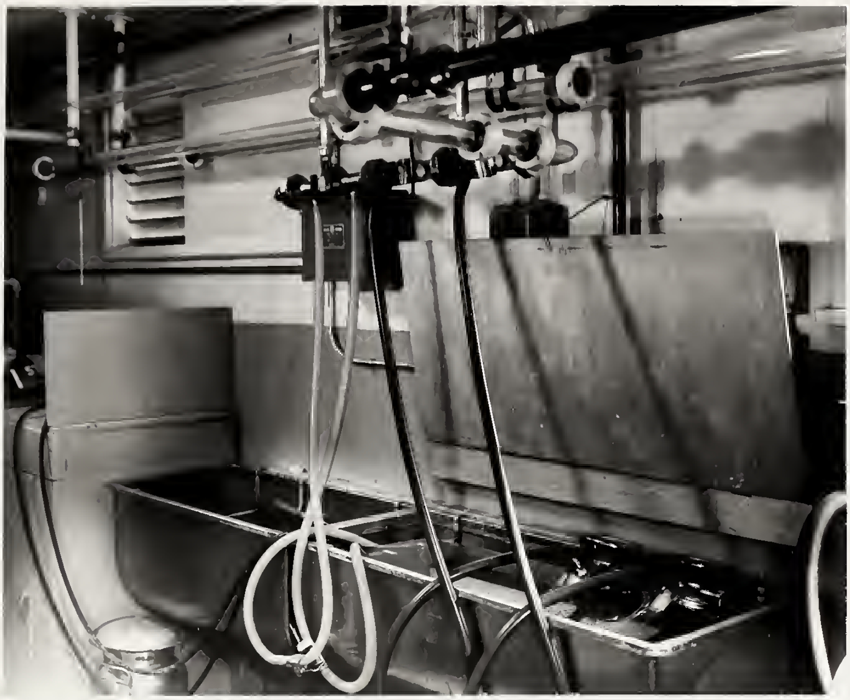

PLATE 10. Lye-EDTA solution tank and rinsing tank immediately before flooding line.

PLATE 11. Rubber seal on De Laval milk inlets for lye flooding.

above CIP methods it was decided to run the circulation cleaning method without disassembling the milking machine clusters or changing the inflations once a week, thus obtaining conditions more closely resembling those of the lye flooding technique. It was also proposed to use this experiment to check if and where the equipment became highly contaminated and also how long the circulation cleaning method could be continued before it became necessary to disassemble the equipment because of contamination which influenced the bacteriological quality of the milk.

On 11/11/63 the clusters and stainless steel section of the pipeline were dismantled, brush-washed and new inflations put on the clusters. One Surge and one De Laval cluster were used in the test, the bacteriological condition of each being assessed by a rinse. The pipeline was tested by the combined rinse and swab method. The results in Table 10 were obtained from tests made following the disinfection procedure before the afternoon milking, except at

the beginning of the trial when the tests were made after the plant had been washed and disinfected following the afternoon milking. Also included in Table 10 are the results of tests on two milkings from the bulk milk tank.

The rinse and recovery solutions were prepared as described previously. The milk samples were tested by the Colony plate count method, with duplicate plates. A simple coliform test using McConkey's broth and fermentation tubes was also made on the milk, using dilutions of 1,10^{-1}, 10^{-2} and 10^{-3}. The results are included in Table 10.

The results for 30/12/63 and 6/1/64 in Table 10 are the totals of separate rinses on sets of inflations, breaker jar, claw piece and long milk tubes. The breakdown of these totals is given in Table 11. The in-line filter holder in the De Laval long milk tube was suspected of being partly responsible for the high counts obtained from the long milk tube as the filter holder contained a 'dead end' (Diagram 3). This 'dead end' was swabbed with a cotton swab on

DIAGRAM 3

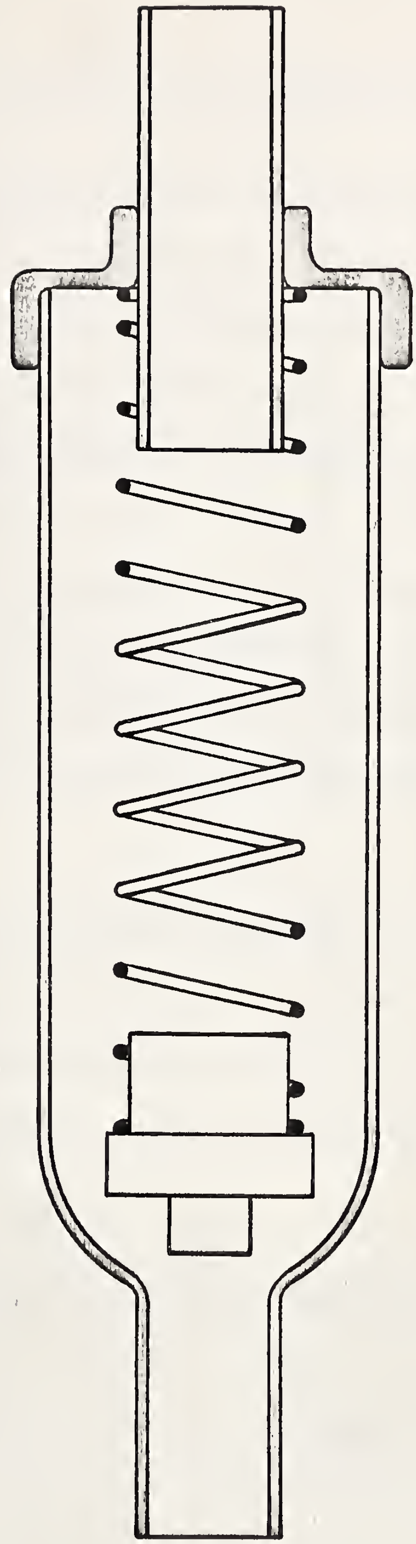

DIAGRAM 3. De Laval filter holder.

8/1/64 and a count of 83×10^3 organisms obtained.

The lye flooding method of C.I.P. was begun on 1/2/64 the clusters having been dismantled and brush-washed and new liners fitted. The clusters, pipeline and milk samples were tested as with the circulation cleaning method. The results for February are shown in Table 12. For the first half of the month the receptacle over the highest point of the line had not been installed so the results from 3/2/64 - 7/2/64 inclusive were obtained without the high section of the line being completely flooded.

The solution was renewed and the clusters dismantled and brush-washed on the 2/3/64. The same inflations were again used for the second month. The results obtained are shown in Table 13.

The De Laval filter holder in the long milk tube was not included in the lye flooding circuit in February but was included in March. It can be seen from Tables 12 & 13 (Column 6) that this filter

TABLE 10. Circulation cleaning of pipeline plant with alkaline detergent followed by sodium hypochlorite

Date	Colony counts from						Coliform present in (ml)
	Pipeline by			Cluster rinses		Milk	
	Rinse (10^3)	Swab (10^3)	Rinse and swab (10^3)	Surge (10^3)	De Laval (10^3)	Count (/ml)	
11/11/63	24	2	26	9	8	-	
12/11/63	32	32	64	4	22	-	
13/11/63	75	50	125	8	198	-	
14/11/63	49	10	59	11	175	-	
15/11/63	11	8	19	3	261	-	
16/11/63	21	2	23	6	194	7,100	0.1
17/11/63	18	3	21	15	198	-	
18/11/63	42	4	46	4	67	9,700	1.0
20/11/63	7	5	12	26	67	9,000	
21/11/63	19	29	48	27	372	-	
22/11/63	54	6	60	9	-	11,600	
23/11/63	38	6	44	9	276	-	
27/11/63	32	7	39	34	600	-	
28/11/63	50	12	62	10	118	7,500	0.1
29/11/63	15	8	23	84	923	-	
4/12/63	27	10	37	86	825	11,500	0.1
5/12/63	49	12	61	200	167	-	
6/12/63	21	4	25	12	400	-	
7/12/63	14	46	60	16	792	-	
9/12/63	18	8	26	2	772	-	
11/12/63	13	10	23	11	1,500	-	
12/12/63	21	11	32	6	192	7,000	1.0
27/12/63	45	2	47	400	1,755	-	
28/12/63	38	23	61	660	1,116	7,200	0.1
30/12/63	-	-	-	252	1,642	6,000	0.1
2/1/64	35	6	41	-	-	-	
6/1/64	-	-	-	181	1,639	-	

TABLE 11. Comparison of rinse results from different types of cluster

Surge Cluster	Colony count (10^3) on		
Date	Inflations	Breaker Jar or Claw Piece	Long Milk Tube
30/12/63	57	35	160
6/ 1/64	4	102	75
De Laval Cluster			
30/12/63	51	51	1,540
6/ 1/64	150	49	1,440

TABLE 12. Results from lye flooding, February, 1964.

Date	Colony counts from						Coliform present
	Pipeline by			Cluster rinses		Milk	
	Rinse (10^3)	Swab (10^3)	Rinse and swab (10^3)	Surge (10^3)	De Laval (10^3)	Count (/ml)	in (ml)
1	-	-	-	46	6	-	
3	113	104	217	190	122	-	
4	26	2	28	1	1	7,100	1
5	912	15	927	41	5	-	
6	169	12	181	34	10	8,000	0.1
7	103	5	108	2	5	-	
17	3,230	23	3,253	31	19	-	
18	161	10	171	42	55	19,300	0.1
19	147	22	169	48	98	-	
20	164	3	167	43	22	16,400	0.1
21	94	4	98	86	60	-	
24	169	8	177	67	83	24,000	0.1
26	-	-	-	-	-	21,000	0.01
27	64	7	71	200	6	-	

TABLE 13. Results from lye flooding, March, 1964.

Date	Colony counts from						Coliform
	Pipeline by			Clusters		Milk	present
	Rinse (10^3)	Swab (10^3)	Rinse and swab (10^3)	Surge (10^3)	De Laval (10^3)	Count (/ml)	in (ml)
3	41	8	49	35	65	5,000	0.1
10	125	3	128	20	152	-	
16	62	13	75	15	395	-	
17	-	-	-	-	-	2,600	1.0
20	500	33	533	210	745	-	
25	194	3	197	46	189	2,200	0.1
26	42	2	44	58	116	-	
29	-	-	-	-	-	5,300*	0.01
30	8,000	500	8,500	50	102	-	
31	3,000	450	3,450	250	1,000	3,500	0.1
1/4/	9,000	2,500	11,500	310	1,100	-	

* Sample held in cooler overnight

TABLE 14. Geometric means of results in Tables 10, 12 and 13

C.I.P. Method		Geometric means of colony counts from					
		Pipeline by			Clusters		Milk
		Rinse (10^3)	Swab (10^3)	Rinse Swab (10^3)	Surge (10^3)	De Laval (10^3)	Count (/ml)
Circulation Cleaning		26.46	8.37	34.83	21.16	301.30	8,300
Lye Flooding	February	170.30	9.63	179.93	33.82	17.29	14,440
	March	402.90	31.96	434.86	65.69	268	3,500

is apparently not properly disinfected by the lye flooding method. As an unsatisfactory result was also obtained from this filter in the case of circulation cleaning (page 95) it would appear that this is an unsatisfactory piece of equipment which requires manual cleaning. On comparing the results of the circulation cleaning method and the lye flooding in March, there appears to be little difference between them in the case of both Surge and De Laval clusters.

With regard to the line, the results of the combined rinse and swab method are considerably lower in the case of the circulation cleaning but the bacteriological quality of the milk is better in the case of the lye flooding, particularly in March though it was worse towards the end of February. When enquiries were made regarding these high counts, it was learned that the milk from 2 cows which had severe mastitis had been in the bulk milk from which the samples had been obtained.

So, taking the results from March as being more

representative of the type of results which can be expected from the lye flooding method it would appear that, although counts from the line are a little higher, a slightly better quality of milk is obtained but it is strongly to be doubted if this latter result has any significance.

The second reason for failing to dismantle and brush-wash the milking machine clusters once weekly with circulation cleaning was to check if and where the equipment became most highly contaminated and if the bacteriological quality of the milk were affected. While the counts from the line did not change appreciably, the counts from both clusters had increased considerably after 6 weeks but this did not affect the count of the milk. The inflations are usually considered to be the most highly contaminated part of milking machine clusters but as can be seen from Table 11 the long milk tube is the main source of contamination in the circulation cleaning period. The most likely explanation for the condition of the liners being superior to that of the long milk tube is because during cleaning and disinfecting

the clusters are hanging inverted inside the wash trough, and during washing the liners receive the highest concentration of detergent solution at the highest temperature for a longer time than any other part of the plant. The same conditions apply during disinfection.

The comparison of the lye flooding procedure with circulation cleaning can perhaps be seen easiest in Table 14 where the geometric means for all types of samples are compared. The safest conclusion that can be drawn is that the lye flooding technique seems to give results as good as with circulation cleaning.

During the second month of lye flooding a check was kept on the concentration of NaOH. The following results were obtained: day 1=4.5%, day 9=3.5%, day 19=2.8%. At this stage it was considered desirable to 'top-up' the solution and 3 gal of 5% NaOH solution was added. This brought the concentration up to 3.2% on day 23, and on day 31 the concentration had fallen to 2.8%.

It would appear desirable to use a higher initial concentration of lye-EDTA in order that its strength would be better maintained over the month and perhaps less draining of rinse water from the plant practised in order to maintain the volume of solution.

DISCUSSION

The two main topics dealt with in this thesis are (a) development of a method for testing the hygienic condition of a pipeline milking plant and (b) comparison of two C.I.P. methods for pipeline milking plants, viz. lye-flooding and circulation cleaning using alkaline detergent plus sodium hypochlorite.

In creating a new method for assessing the bacteriological condition of milking equipment, reproducibility of results from similarly treated surfaces is the main consideration, even more so than the actual number of organisms removed from the milk contact surfaces. The types of surfaces involved and the inaccessibility of these surfaces because of the design of the equipment are problems particularly with pipeline milking plants.

The types of surface with which milk comes into contact in a pipeline milking plant are glass, stainless steel, plastic and rubber. The rubber surfaces

are the most difficult to clean as the treatment which milker rubberware receives does not deal adequately with the small pores and crevices in the rubber surface; this applies especially to the inflations. The rubber milk contact surfaces in a pipeline milking plant are the inflations and the long milk tubes. The gaskets between sections of pipe are of a synthetic material (neoprene) which may also cause bacteriological problems unless they are flush with the internal surface of the pipe and unless the sections of the pipe are fitted tightly against the gasket. The inaccessibility of the milk contact surfaces is another problem as it is desirable to disassemble as little of the equipment as possible for testing purposes.

The results of the circulating rinse of the plant (Table 2) may provide a partial explanation of the high counts obtained from bulk milk tanks even when the count of the milk taken directly from the cow and the results of an ordinary bacteriological rinse test on the equipment are low. It would appear from the comparison of the rinses and

swabs that vigorous scrubbing action and prolonged contact is required to remove many of the micro-organisms.

The results of the swabs of pipeline and clusters after washing the plant for 20 min with 0.5% tri-sodium phosphate and disinfecting with 200 p/m of hypochlorite for 3 min show on calculation a total of 371,000 plus 36,000 for the whole of the internal surface of the pipeline assemblage. Assuming 70 gal of milk was obtained at a milking and 407,000 organisms added to the milk, this would increase the count of the milk by a little over one organism/ml. A high average bacterial count of milk direct from the cow in this work would be approximately 1000/ml. Clearly the estimation of the population of the pipeline afforded by the swab method would not contribute any appreciable increase, to yield numbers of bacteria such as are normally found in bulk milk. The results of rinsing the pipeline and clusters for 30 min and 15 min respectively indicate a much greater number of micro-organisms in the pipeline. A count of 20×10^6 plus 25×10^6

(from Table 2) would only yield, according to the calculations above, 140 bacteria/ml of milk. However, the vast difference of the two estimates of the bacterial contamination of the pipeline given by these two methods suggests that a larger volume of warm fluid in contact with the pipeline for a longer period (warm milk) might remove an even higher number of bacteria. It is very likely that the combined rinse and swab (using sponge) of the pipeline has also given an under-estimate of the contamination of the pipeline.

It is probable also that the swab contact method on the bulk milk tank would remove only a small proportion of the organisms and that the prolonged contact with the milk which is agitated would remove many micro-organisms from the surface of the tank, further increasing the count of the milk.

With the comparison of static rinse and swab methods of testing inflations (Table 3) it was found that generally a higher count was obtained by the rinse method but not appreciably so. However, Johns & McClure (1961), in a comparison of swabs and pul-

sating rinses on inflations, found large differences, the rinse results being generally very much higher than those from the swabs. Thus, pulsation of the inflations during milking is another possible source of micro-organisms. A further possibility is the breaking up of clumps with the agitation of the milk in the bulk milk tank.

This disparity between numbers of micro-organisms obtained by swabs and rinses, and the numbers found in bulk milk was commented on recently by Jackson (1963).

If the difficulties with the alginate wool swab for testing the line had been overcome, this would probably have been a suitable method, as all the organisms removed from the line would have been recovered and the chance of contamination from the air reduced to a minimum. The use of a blender for the recovery solution which would disintegrate a swab tied with alginate wool thread might be advantageous. Where a sponge is used for swabbing, there is a possibility that some of the organisms may be washed into the interior when the sponge is placed in the recovery

solution because most of the water has already been removed from it by vacuum. While the combined rinse and swab method may remove only a small proportion of the organisms from the line, nevertheless it gives an indication of failure in washing and disinfecting the line.

The lye flooding method of cleaning in place appears to be as good as the circulation cleaning method. Although the equipment rinse counts were a little higher, the counts in the milk were a little lower, though perhaps not significantly so.

As the sponge used for testing may have a cleaning effect on the line, it would be desirable to make a comparison of a month's run without any testing with a month during which the line was tested frequently. This should give an indication of any possible cleaning effect of the sponge. Another point which should be considered is the possibility of using a sponge for drying the line immediately before milking as a standard practice. With circulation

cleaning the line was drained of disinfectant by means of air being drawn through it for 3 min. With lye flooding the draining of the line depends on the slope of the line (1.5 in/10 ft). Thus, the use of a sponge for drying might allow milking to begin sooner after the rinsing procedure. It would also lessen the risk of milking beginning before the line had been properly drained; further, the sponge might have a possible cleaning effect.

The water used for rinsing the milk and sodium hydroxide from the line may also cause problems. At the University Farm, mains water with a colony count of 0-2/ml is used. With a farm having a water supply of unsatisfactory bacteriological quality, some method of disinfection would be required before it could be used for rinsing the line.

Further study is required before it can be stated with certainty whether the lye flooding technique will give satisfactory results as it must also be tested under summer conditions. There is no appar-

ent reason why it should not prove satisfactory as the immersion cleaning technique has been found to give satisfactory results under summer conditions in Canada.

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